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Preface

Speciation is a fundamental evolutionary process, knowledge of which is critical for understanding the origins of biodiversity. Genomic approaches are an increasingly important aspect of this research field. We review current understanding of genome-wide effects of accumulating reproductive isolation and of genomic properties that influence the process of speciation. Building on this work, we identify emergent trends and gaps in our understanding, propose new approaches to more fully integrate genomics into speciation research, translate speciation theory into hypotheses that are testable with genomic tools, and provide an integrative definition of the field of speciation genomics.

Introduction

Major insights into the genetics of speciation have come from a number of approaches (Box 1), ranging from the mapping of individual genes causing **reproductive isolation** (RI) to the characterization of genome-wide patterns of differentiation, and from quantitative genetic approaches to admixture analyses associating phenotypes with reduced **gene flow** between populations[1-3]. These empirical approaches have a long history, starting with the work of Dobzhansky[4] and Muller[5]. Theoretical understanding of the genetics of speciation has advanced markedly[6-10]. However, the deluge of empirical data coming from **next generation sequencing** (NGS), along with the emergence of new analytical approaches, necessitate the integration of this theoretical work to strengthen the conceptual foundations of the nascent field of **speciation genomics**. Such integration will help elucidate the relationships between evolutionary processes and genomic divergence patterns on the one hand, and between genomic properties and speciation processes on the other, and it will help unify research on the ecological and non-ecological causes of speciation.

In this review, we first discuss areas in which genomic approaches have begun to make important contributions to speciation research (Box 1), for example by elucidating patterns and rates of genome-wide divergence, improving our understanding of the genomic basis and evolution of intrinsic and extrinsic reproductive barriers, and identifying mechanisms by which different barriers become genomically coupled. We also highlight areas that would benefit from further attention; these areas include the distributions of **locus effect sizes, pleiotropy** and genomic constraint. We conclude by discussing how NGS data and innovative population genomic analyses could contribute to further progress in integrating these study areas into a more comprehensive and coherent understanding of the genomics of speciation.

The evolution of reproductive barriers: Theory and classical evidence

In line with others[1, 3], we define speciation as the origin of reproductive barriers among populations that permit maintenance of genetic and phenotypic distinctiveness of these populations in geographical proximity. The origin of reproductive barriers can either be initiated by **divergent selection** (that is, "ecological" or sexual selection creating **extrinsic reproductive isolation**), or by the evolution - through genetic drift, as an indirect consequence of selection or through genomic conflict - of genetic incompatibilities that cause **intrinsic reproductive isolation** (Box 2). Studying the accumulation of intrinsic isolation has a strong tradition in evolutionary biology[1, 11]. Yet, most recent population genomic studies of divergence across the genomes of incipient and sister species have

investigated cases of putative **ecological speciation** and have focused on divergent adaptation and extrinsic isolation (but see[12] discussed below).

Extrinsic postzygotic isolation arises as a consequence of divergent or **disruptive** natural **selection** when the viability or fertility of migrants or of individuals with intermediate genotypes is reduced[2]. Prezygotic **sexual isolation** and also extrinsic postzygotic isolation, when hybrids have reduced mating success[13], may evolve as a consequence of divergent sexual selection[3, 14] which is often, but not always, mediated by differences in environments[15, 16]. Prezygotic sexual isolation and extrinsic **postzygotic isolation** are, hence, dependent on genotype-environment interactions in the wider sense (where mating partners are part of the external environment). In contrast, intrinsic postzygotic isolation is independent of the external environment. Consequently, different types of genes and gene networks and different evolutionary processes may be involved in generating these classes of isolation. Extrinsic postzygotic isolation and sexual isolation can evolve rapidly[17], and they often interact with each other[16] and with the evolution of intrinsic postzygotic isolating barriers[18] (Box 2). Selection can initiate speciation in situations with and without gene flow between populations, while intrinsic incompatibilities are less likely to accumulate when gene flow is present[6]. This being said, adaptive divergence and ecological speciation are not the same. Divergent adaptation alone rarely causes sufficient reproductive isolation to allow the accumulation or persistence of species differences in geographical proximity: this typically requires the evolution of **prezygotic isolation**[1, 3] (Box 2), although it is possible that this varies between major taxonomic groups such as insects versus vertebrates or plants.

The available evidence suggests that negative epistatic interactions, so called **Bateson-Dobzhansky-Muller incompatibilities** (BDMIs, or often just referred to as DMIs), are the most frequent cause of intrinsic postzygotic isolation[1, 19-21]. However, other mechanisms, including **underdominance**[22] and gene duplication, transposition and gene loss[23-25] may also cause intrinsic postzygotic isolation. The time course of the accumulation of DMIs is not well understood[19, 26-28], and rates may vary among taxa and among mechanisms underlying DMI evolution[19]. DMIs were long thought to arise either as a consequence of genetic drift, as a result of stochastic deactivation of gene duplicates[29] or as a by-product of ecological selection[30]. However, theoretical considerations, such as the slow pace of neutral accumulation of barriers[31], and early empirical evidence for positive selection on loci contributing to incompatibilities[32], suggested that drift was unlikely to be a common source of incompatibilities. Recent observations suggest instead that **intragenomic conflict** may be a common mechanism driving their evolution[20, 33-35] (Fig. 1), as originally proposed in 1991[34, 35]. Genomic conflict may arise from competing interests of males and females[36], from **meiotic drivers**[37, 38], mobile elements[39, 40], or other selfish genetic elements and their suppressors, and from competing interests between organellar and nuclear genomes[41, 42]. **Sexual conflict** is thought to drive the evolution of new sex chromosomes[43, 44], and empirical observations suggest sex chromosome turnover has a role in the evolution of reproductive isolation[45,46].

The different evolutionary mechanisms underlying the build-up of extrinsic and intrinsic postzygotic and of prezygotic isolation suggest that genomic signatures will also be distinct. The genomic architecture of extrinsic isolation is likely to resemble that of adaptive population divergence, and be diverse and scattered across multiple regions in the genome (see below). However, there are theoretical arguments and empirical evidence for spatial clustering of sites under selection in the genome when adaptive evolution proceeds under prolonged bouts of divergent selection with migration or recurrent hybridization[47]. For intrinsic isolation, incompatibility factors driven by genomic conflict are expected to accumulate in genomic regions of reduced recombination where linkage disequilibria between **distorter loci** and **responder loci** can become established[48, 49]. Sex chromosomes are particularly susceptible to the accumulation of incompatibility factors derived from genomic conflict because sex chromosomes are constantly in a battle over segregation, whereas only small and tightly linked autosomal regions are in conflict with their homologs[34]. At the same time,

there will be particularly strong selection for suppression of sex-linked distorter loci because they tend to bias sex ratios[50, 51]. The genomic architecture of certain types of prezygotic isolation may also be influenced by regions of reduced recombination around sex determining loci[52] or sex chromosomes[53], particularly when sex-linkage resolves sexually antagonistic effects of sexual selection[54]. Alternatively, prezygotic isolation loci may accumulate near extrinsic ecological isolation loci (see section below, "***Genomic coupling of reproductive barriers"***). All of these signatures must be distinguished from background patterns of genetic diversity and divergence that depend on the populations' history of genetic drift, gene flow, background selection and episodes of positive selection unrelated to reproductive isolation.

Looking for signatures in the genetic architecture of reproductive isolation has a long "pre-genomic" history ([55, 56]). However, there has been a historical disconnect between research programs focused on intrinsic isolation, which have typically concentrated on later stages of speciation[20, 57], versus extrinsic postzygotic isolation and prezygotic sexual isolation at early stages of speciation[2, 30,15, 16]. Because of this disconnect, comparing the rates of evolution of components of reproductive isolation, and their relevance to speciation, is currently a challenge. Where rates have been compared in the same taxon using "pre-genomic" methods[11, 58-60], the data suggest that prezygotic and extrinsic postzygotic isolation often evolve faster than intrinsic postzygotic isolation, consistent with expectations from classical theory[61]. Genome-wide data will now permit testing of this pattern with a tremendous increase in resolution.

Genomics and the speciation continuum

Once speciation is complete, populations accumulate differences due to mutation and genetic drift as well as ongoing selection. Reproductively isolated species, therefore, often differ in traits that evolved under ecological selection and others that evolved under sexual selection, and may also have intrinsic incompatibilities. A central task of speciation genetics is to reconstruct the sequence in which these different barriers originated so as to distinguish between causes and consequences of speciation. To achieve this, one would ideally take an unbiased view of the entire genome at all stages of the same speciation process. However, speciation can rarely be studied in real time in natural populations of sexually reproducing multicellular organisms. Estimates of variation among loci in the timing and magnitude of gene flow could help determine the order in which reproductive barriers emerged, but such inferences are challenging and current methods are not accurate enough for this purpose[62]. However, by integrating case studies of closely related taxa that vary in their extent of divergence (the "speciation continuum"), inferences can often be made about the chronology and significance of different factors and processes at play.

Investigations of this **"speciation continuum"** have made important contributions to speciation research[63, 64] and this approach is being adopted in NGS-based genome and transcriptome scan studies of speciation. The major questions being addressed are the extent to which divergence at different stages in speciation is either localized in the genome (the "island view") or widespread, the extent to which heterogeneity in divergence can be attributed to selective processes versus genetic drift, the sources of selection, whether genomic divergence tends to follow a common trajectory as it proceeds along the speciation continuum, and how all this is affected by the extent of geographical isolation. A recently much cited scenario for speciation without strong geographical isolation, derived from earlier models[65, 66], involves an early stage of divergence where differentiation is limited to a small number of loci (islands) under strong divergent selection. Gradually, these regions would grow through the process of **divergence hitchhiking**, and eventually the effective migration rate would become reduced globally across the genome fostering genome-wide divergence ('genome hitchhiking')[67, 68].

Genome scans of ecological speciation

Several NGS-based **genome scans** of the speciation continuum have found surprisingly variable patterns of genomic divergence. It appears that incipient species can quickly accumulate substantial divergence, even in the presence of gene flow (Fig. 2). However, whereas in some examples - such as *Heliconius* butterflies[69], *Helianthus* sunflowers[70], and poplar trees[71] - divergence between **parapatric** ecotype populations is limited to a few large genomic regions, in others it is widespread across the genome[72-75]. NGS-based genome scans of **sympatric** sister species have generally reported genomically widespread and highly heterogeneous divergence that varies on a very local scale[75-81]. Few studies have looked for evidence of divergence hitchhiking and the available results are inconsistent[69, 76, 82]. Genome-wide average $\mathbf{F_{ST}}$ often increases as phenotypic divergence increases[80, 83] but divergence seems to remain heterogeneous across the genome for a very long time, potentially due to repeated episodes of interspecific gene flow even after RI has become strong[84, 85]. The first generation of NGS-based population-genomic studies of ecological speciation has therefore shown that ecological selection can cause strong isolation of small genomic regions between diverging populations, and that when RI is strong enough to permit persistence of incipient species in sympatry, many unlinked regions typically experience significant isolation.

So where does the heterogeneity in genomic divergence come from? It is commonly inferred to result from locus-specific differences in the effects of divergent selection and gene flow. Indeed, genome scans have shown strong isolation at genomic loci that were known to be under divergent selection[64, 69, 70, 72, 74]. However, caution is warranted as different evolutionary processes can leave similar signatures in the genome. Heterogeneous genomic divergence is sometimes also observed between allopatric populations of the same species in the absence of any current gene flow[76, 86, 87] (Fig. 2). Indeed, many studies assume ongoing gene flow between species, even though stochastic variation due to recent **coalescence** times and **incomplete lineage sorting** can similarly lead to low divergence and high heterogeneity, particularly when in combination with selection[88, 89]. Statistical methods are available to distinguish divergence in isolation from divergence with gene flow, and these methods are increasingly being applied to genome scale datasets (reviewed in [90]; Box 1).

Even in the absence of selection, divergence is expected to vary due to the stochasticity of genetic drift and the complexities of population history, and this variation can be enhanced by confounding effects of genomic heterogeneity[91]. In particular, regions of low recombination and/or high gene density often show reduced intra-specific diversity, which inflates relative divergence as measured by F_{ST} or D_a[88]. This can result from background selection against deleterious mutations[92], intraspecific selective sweeps (in allopatry)[88] or even a direct influence of recombination on genetic diversity[93]. Disentangling these processes is challenging[94]. Some have suggested correcting for recombination rate in interpreting F_{ST} patterns[83]. Others have suggested that absolute divergence measures such as $\mathbf{D_{xy}}$ are more robust to diversity artefacts[95], especially when corrected for local mutation rate[96]. It seems unlikely that any single parameter will reliably disentangle divergent selection and gene flow from neutral processes. Good knowledge of the geographical context of population divergence will help, but distinguishing between hypotheses of speciation with gene flow, secondary contact and incomplete lineage sorting will often require new, parameter-rich modeling approaches[90].

Adaptive divergence has been shown to accumulate preferentially in regions of low recombination[97], including the centers of chromosomes[83], the vicinity of centromeres[98], inversions[74] or often (but not always[12, 71]) on sex chromosomes[98-100]. Heterogeneity in genomic divergence seen in allopatry might also result from **gene-flow-selection balance** that has occurred in the past[47, 76]. Finally, the assumption that the baseline F_{ST} reflects neutral divergence may be violated in cases where divergent selection is pervasive and multifarious, and this would bias against the detection of the signature of selection[81].

Evidence for repeated divergence of the same genes or genomic regions across replicate pairs of species or environmental contrasts already provides strong evidence that these regions are indeed involved in adaptation and/or RI[72, 74, 85, 97, 101-103]. Detecting such parallel divergence may require dense sampling of genomes or transcriptomes because the highest levels of repeatability may be observed at the scale of genomic regions rather than individual genes or SNPs[97]. In this case, the repeatability in the heterogeneity of genomic divergence may be due at least in part to shared genomic heterogeneity in recombination and mutation rates rather than parallel adaptive divergence, but the shared genomic structure may facilitate the repeated accumulation in the same genomic regions of adaptive differentiation[97]. Another approach involves combining classic **cline** theory with genome-wide analyses, allowing measurements of the strength of selection at specific loci[79] (Box 1). In the future, parameter-rich **coalescent** models of divergence with gene flow fitted to genomic data may be able to account for the heterogeneity of demographic history across the genome when seeking to identify genomic regions with reduced gene flow[104, 105]. Finally, genome scans combined with manipulative selection[81], QTL mapping[82, 106], candidate gene mapping[72, 74] and **admixture mapping**[79, 107-109] can be used to investigate whether divergent genomic regions contain loci contributing to RI.

Several recent studies have found a contribution of ancient alleles to recent divergence, as exemplified by stickleback[74, 110], cichlids[77, 111], *Rhagoletis* flies[112] and *Heliconius* butterflies[113]. Ancient alleles are identifiable due to the accumulation of many substitutions or sharing over wide spatial or taxonomic ranges. The sources of such ancient allelic variation can either be **standing genetic variation**, or **hybridization**[114]. Distinguishing between these hypotheses is difficult in practice due to the challenges of distinguishing **incomplete lineage sorting** from hybridization[90] (Box 1). The balance of evidence from NGS data implies **introgressive hybridization** rather than standing variation as the source of ancient alleles in most of the above cases. Speciation in these cases might have been facilitated by hybridization providing genetic material for adaptation and reproductive isolation in the face of gene flow, which puts a new twist on an old idea [1]. Future research combining genomic and ecological approaches should test this hypothesis further.

Genomic divergence and intrinsic isolation

Many studies have investigated DMI genes in strongly isolated species, but in many cases it remained unclear if the **fixation** of the underlying mutations was a cause or a consequence of speciation[20, 57]. Regardless of whether identified DMI alleles are the first step in the origin of reproductive isolation, a striking pattern to emerge from recent work is that they have evolved under strong positive selection rather than genetic drift and that **genomic conflict** is often implicated as the source of this selection. For example, one study identified *Ovd*, an X-linked gene that underlies both hybrid male sterility and sex-ratio distortion in crosses between *Drosophila pseudoobscura pseudoobscura* and *D. p. bogotana*[51]. Another example is a recent analysis that found strong evidence for ongoing positive selection within *Drosophila mauritiana* in genes that have diverged between this species and its closest relatives and that are known to be involved in genomic conflict[12]. Two pronounced polymorphism troughs on the X chromosome were centered on a pair of genes that cause sex-ratio distortion within *D. simulans*, and on *Odysseus*, a rapidly evolving homeobox gene that was known to cause male sterility in *D. mauritiana* x *D. simulans* hybrids[32] and may be involved in genomic conflict. These are two candidate cases of speciation by conflict-driven DMI evolution.

Genomic coupling of reproductive barriers

The build-up of associations between several traits or loci involved in RI strengthens the total barrier to gene flow between diverging populations, and is therefore important for the evolution of strong reproductive isolation[115, 116]. Such **genomic coupling** can involve any pre- or post-zygotic barriers[117]. Deviations from linkage equilibrium between barrier loci can initially be generated by new mutations arising on a particular genetic background, or by genetic drift during divergence with limited gene flow. Coinciding barriers may, for example, arise through secondary contact between divergent

298 populations, through the evolution of DMIs as an incidental by-product of divergent selection[118], or
299 via hitchhiking of intrinsic incompatibility alleles with divergently selected alleles, as has been shown
300 for heavy-metal adapted populations of monkey flowers[119]. However, for barrier coupling to be
301 important in speciation, coupling has to be maintained or even strengthened in the face of gene
302 flow, and this typically requires divergent selection[6].

303 Selection is expected to favour the coupling of barriers if this leads to an increase in mean fitness. In
304 theory this can involve multiple intrinsic barriers (like DMIs)[120, 121] or intrinsic and extrinsic
305 postzygotic barriers as well as sexual and other prezygotic isolation traits. Across an **ecotone**,
306 multifarious extrinsic selection can assemble and maintain many coinciding clines at loci involved in
307 adaptation[122], and these can become coupled with sexual isolation traits[123] and with DMIs[18,116, 124].
308 Selection can also directly favour the evolution of increased prezygotic isolation, as in the case of
309 **reinforcement**[125]. Finally, sexual conflict can couple intrinsic postzygotic and prezygotic sexual
310 isolation because DMIs driven by sexual conflict and genes underlying sexual traits or preferences
311 expressed only in one sex may both accumulate on sex chromosomes[53, 126]. Consistent with these
312 expectations, loci for plumage colour, mating preferences and intrinsic postzygotic incompatibilities
313 are coupled on the *Z* chromosome in flycatchers[52] and Gouldian finches[127, 128]. Similarly, loci for
314 behavioural isolation and hybrid male sterility are coupled on the *X* chromosome in a species pair of
315 Japanese stickleback[45].
316
317 Because recombination tends to break up gene associations, genomic architectures that eliminate or
318 decrease recombination are expected to facilitate coupling, and hence speciation[129]. Most
319 prominently, recombination will affect neither associations among traits that are pleiotropically
320 influenced by the same allele, nor **'one-allele' mechanisms**, where the presence of the same allele in
321 different genetic backgrounds confers RI[130]. One-allele mechanisms do not leave a population-
322 specific signature in the genome at the primary isolation locus but they should be detectable as
323 **sweeps** shared by both diverging populations if they arise during speciation (as for instance if an
324 allele for imprinting on the phenotype of the father spreads across two incipient species that were
325 connected by gene flow). Despite the theoretical expectation that 'one-allele' mechanisms evolve
326 more readily during speciation with gene flow than other types of barriers[6, 16, 130], we are not aware
327 that the predicted genomic signature of shared sweeps at isolation loci has yet been detected in any
328 case. Revealing such a signature would be a strong contribution of speciation genomics to
329 demonstrating a classical prediction of speciation theory.
330
331 Loci underlying **'two-allele' mechanisms** are expected to be concentrated in regions of reduced
332 recombination. Recent genomic studies have observed genomic architectures that eliminate or
333 reduce recombination between traits involved in RI: There is evidence of synergistic pleiotropy in
334 **multiple-effect or "magic" traits**[16, 131-133], and multiple genes underlying isolating traits have been
335 found together in inversions[134-136], on sex-chromosomes[45, 52, 128] and also in otherwise tight physical
336 linkage[119, 137], including mating traits and mating preferences in cases of speciation with gene flow[138].
337 These data also provide some evidence that reinforcement of prezygotic isolation is facilitated by
338 linkage, as in flycatchers[139], or by pleiotropy, as in phlox[132]. In other cases reinforcement might be
339 constrained[140] where loci are not linked and where there is extensive gene flow. However, recent
340 genomic studies have also provided empirical examples of coupling between unlinked loci in fully
341 **sympatric** hybridizing species[77] and especially in **hybrid zones**, where clines at many unlinked loci
342 often coincide, although it is not always clear exactly how these loci are implicated in RI[141]. Unbiased
343 whole-genome re-sequencing data and genome scans from diverging populations, coupled with
344 methods to reduce bias from NGS data[142] and with mapping of isolation traits, are needed to test the
345 generality of these patterns.
346
347 ***Effect sizes and pleiotropy***
348 A key question, with a long history[55, 143], is whether speciation is typically initiated by divergence at
349 few loci of large and possibly pleiotropic effect or by divergence at many loci with small and additive

effects[133, 144]. The distinction is important because it will affect how speciation is constrained by the availability of suitable genetic variation, and will also affect how likely it is that selection or genetic drift may overcome gene flow. On their own, F_{ST} estimates from genome scans tell us little about the effect sizes of individual alleles on phenotypes, fitness or RI[107] (Fig 3). With regard to fitness, Fisher's geometric model predicts that the probability that a mutation is favourable decreases exponentially with mutational effect size, hence we expect few alleles of large positive fitness effect but many of small effect[145-147] (but see[148]). However, this prediction does not take into account standing genetic variation, gene flow or changing environments. When those factors are considered, the predictions change[47, 147, 149] and may even reverse[150].

Speciation with gene flow may require divergent or disruptive selection to be concentrated on a small number of regions in the genome that also have large effects on RI[6]. Theoretically expected distributions of effect sizes in terms of RI (rather than fitness) may be different for different classes of isolating barriers, but current data are equivocal (Fig. 3b). For example, mapping hybrid inferiority in natural environments for *Arabidopsis* has shown RI to be due to many genes with moderate effects[151]. In contrast, hybrid inviability in *Mimulus guttatus* is a consequence of two linked loci of major effect[119]. Predictions about the distribution of effect sizes expected for genes that underlie DMIs are also generally lacking, partly because effect sizes depend on mutation order and the extent of background genomic divergence. Traits governing prezygotic isolation, and especially sexual isolation (Box 2), are likely to have large effects on RI because they directly influence mating or fertilization patterns[1, 6, 16, 152-154]. To test this prediction with genomic scale data, existing quantitative genetic, mapping and candidate gene studies[45, 109, 111, 128, 138, 155-157,158, 159] should now be followed up by NGS-based genome scans assessing RI around these loci[107].

Recently identified large-effect alleles involved in adaptation and speciation with gene flow, are often highly **pleiotropic** (e.g., *Optix* in *Heliconius*[160] and Ectodysplasin [*Eda*] in sticklebacks[161], although we lack estimates of the effect *Eda* has on RI or fitness). Such alleles may be rare among newly arising mutations but alleles with synergistically pleiotropic effects may be more common in standing genetic variation. Recent theory suggests that large-effect or pleiotropic alleles may be favoured by selection during evolution in gene-flow-selection balance, and hence eventually become enriched in taxa with divergence and gene flow[47].

Genomic constraint

The flipside of the coupling problem is that genetic correlation between traits as a result of pleiotropy or tight linkage may also constrain speciation. With new population genomic data revealing divergence in many regions of the genome early in speciation, there is an opportunity to unite population genomics with a quantitative genetics perspective on the evolution of polygenic traits during speciation. In quantitative genetics terms, standing genetic variation is quantified by the **G-matrix** of additive genetic variance and covariance[162]. **G** may indicate potential constraints on adaptive evolution that affect the response to directional selection[163, 164], as well as constraints on genetic drift[165]. Tests to detect the impact of selection on G are available (e.g.[166]). Divergence among populations is biased along axes with greater genetic variation and covariation and constrained along axes with little variation or covariation[164, 167, 168]. Importantly, however, genetic constraints are not only negative. Genetic covariation may align with **correlational selection**[169, 170] and, as discussed above, pleiotropy can couple adaptation to RI. It is not known how genes of major effect, versus the traditional assumption of many genes of small effect, influence the structure of **G**[171], and how higher moments of the distribution of genetic variation and covariation affect the response to divergent selection[172]. These questions can now be addressed with genomic methods, such as directly estimating **G** in outbred populations using NGS data[173]. A different approach is to estimate the genetic variance–covariance matrices for gene regulatory networks from gene expression data. Analyzing genomic data in a quantitative genetics framework in this way will illuminate how genomic constraint affects speciation[174].

Studying effects of hybridization is one promising application. Beyond being a source of allelic variation, hybridization may facilitate evolution and perhaps speciation by releasing populations from constraints caused by genetic correlations. While empirical evidence has accumulated that suggests that selection alters genomic architecture[169, 175], the role of gene flow in aligning **G** with the direction of divergent or disruptive selection has rarely been investigated[150]. The emerging consensus that hybridization frequently introduces adaptive variation[18] calls for empirical studies in this area. We predict that hybridization will influence speciation not only by generating novel and **transgressive phenotypes** but also by aligning **G** with the axis of divergent selection (Fig. 4a). Even when early generation hybrids are maladapted, hybrid populations may over time benefit from increased evolvability[176]. Hybridization may alter patterns of genetic covariance much faster than is possible by selection alone, and may lead to bursts of evolutionary diversification and speciation[114, 177] (Fig. 4b-d). Genomic methods can now be used in assessing these hypotheses in several ways, such as direct estimation of **G** in both parental and hybrid natural populations and through association or **admixture mapping** of loci contributing to novel adaptive phenotypes in hybrid populations[108].

Speciation genomics: towards a synthesis

Speciation can proceed in many different ways, but these can be grouped in terms of drivers (drift and different types of selection), causes (extrinsic environment-dependent versus intrinsic environment-independent) and stage in the life cycle (postzygotic or prezygotic) of reproductive isolation, resulting in two major classes that are at least in theory quite distinct (Box 2). In one, RI is initiated by extrinsic selection, in the other by intrinsic incompatibility. Analysis of NGS data has begun to shed light on the signatures of these processes in the genome. Both of these classes of processes can generate reproductively isolated species in allopatry, but parapatric and especially sympatric speciation are constrained to situations where divergent natural and/or sexual selection overcome the homogenizing effects of gene flow[1, 6]. Whether speciation in such scenarios can proceed depends on the strength of selection[2, 6] and the genetic architecture of adaptation and reproductive isolation[76, 122]. Speciation driven by genomic conflict is much less likely to be initiated in the presence of gene flow because selfish genetic elements may then spread across populations and thereby prevent or slow down the accumulation of conflict-driven DMIs[178]. However, it remains possible that relatively brief periods of **allopatry** are sufficient for the origins of conflict-driven DMIs. Although DMIs may be removed by selection after **secondary contact**, they may, in theory, facilitate speciation if they become coupled with other components of RI before they are purged[116, 179]. How often this happens is unknown.

These principles are not new[1], but they can and should now be examined with much greater resolution using genomic methods. Although speciation genomics is clearly still in its infancy, a few trends are emerging from the first generation of NGS-based genome scans, particularly in relation to non-allopatric speciation: The available evidence suggests that divergence can be genomically widespread very early in speciation, and may generally be so in species that coexist in full sympatry[74-77, 80], whereas it can be restricted to very few islands of divergence in parapatric ecotypes[69, 70]. Perhaps **multifarious divergent selection** or genomically widespread selection is important to generate sufficient RI to permit maintenance and perhaps buildup of genetic differentiation in sympatry. More data are now needed to confirm this intriguing pattern.

Some genomic regions that are divergent between incipient and sibling species in geographical proximity contain genes with large effects on adaptation and pleiotropic effects on prezygotic isolation. The alleles at several such loci have turned out to be ancient variants that were present as standing variation or were brought together by hybridization in the ancestors of emerging species

pairs[99, 111, 112]. Although it is premature to draw strong conclusions, this may turn out to be another emergent feature of speciation with gene flow. We expect effect sizes to be larger, antagonistic pleiotropy to be less frequent and synergistic pleiotropy to be more frequent in ancient alleles that have been honed by selection over time than in alleles arising newly through mutation. We hypothesize that substitution of such ancient alleles at major effect loci has the potential to reduce gene flow quickly, to the point where substitutions with smaller effects at other loci can also spread. Genome scans of divergence very early in the speciation continuum (at low overall RI, Box 2) should allow explicit tests of these hypotheses.

Alternative mechanisms and geographical modes of speciation make different predictions for patterns in genomic data. Specifically, we predict that speciation due to conflict-driven DMIs involves greater divergence at centromeres and sex chromosomes, and so these regions should bear signatures of selective sweeps. Divergence under ecological selection may be more widely distributed across the genome, and sweeps at individual loci less pronounced. The available data are consistent with these expectations, although theory predicts accumulation of genes for ecological divergence in regions of low recombination when selection is antagonized by gene flow[129]. Divergence by sexual selection may be concentrated on sex chromosomes[52], but support for this prediction is not always found and predictions vary with the sex determination system[54]. Many more population genomic studies of divergence in a wider range of taxa and across a greater range of points along the speciation continuum are needed to test these predictions further. Speaking more broadly, future work should seek to determine to what extent different evolutionary mechanisms and geographical modes of speciation can be distinguished based on genomic data and, in turn, the extent to which genomic features can predict the modes and mechanisms of speciation that apply to a given evolutionary lineage.

Taxonomic variation in the propensity for speciation without geographical isolation is prevalent[180] and it will be interesting to learn if variation in genomic architecture explains some of this. Whether selection can overcome gene flow depends, besides the total strength of selection, on the number of genome regions targeted by selection, on the rate of recombination between them, and on the extent of pleiotropy. When analyzed in conjunction with ecological data, genomic data therefore hold promise to help explain why non-allopatric speciation occurs readily in cichlid fish, whitefish, stickleback, *Rhagoletis* flies, *Heliconius* butterflies, *Coprosma* shrubs[181] and some other taxa, but is not reported in the majority of others. This combination of approaches may also help more generally to explain why some taxa undergo speciation and accumulate species diversity a lot more readily than others. Answering such questions will also facilitate an understanding of larger-scale patterns in species diversity (Box 3).

Population-genomic studies that explicitly compare rates of evolution and the genomic distribution of prezygotic, extrinsic postzygotic and intrinsic postzygotic barriers to gene flow have yet to materialize. We believe that such studies hold considerable promise to overcome old dichotomies in speciation genetics. Because the discovery of DMIs used to be laborious, we cannot yet answer the question how often DMIs are caused by conflict, ecological selection or genetic drift. This too will hopefully soon change as genomic data allow the identification of DMI loci at an increasing pace[12, 26] (Box 1).

A still missing part of a synthesis in speciation genomics is the integration of evolutionary developmental biology. Insights from this field make several relevant suggestions for speciation genomics[182]: First, mutations in coding sequences may more often have pleiotropic effects than those in *cis*-regulatory regions. Second, pleiotropy will be more common when selection targets genes with central roles in gene regulatory networks, and many morphological developmental genes are in such positions. Third, because of the first two predictions, morphological evolution may often be constrained to take place through changes in *cis*-regulatory mutations, whereas physiology may

be more free to evolve through coding mutations. These predictions make for interesting yet little explored connections between some of the above discussed questions in speciation research and the debate about the prevalence of coding versus *cis*-regulatory mutations in evolution[182, 183]. Possible ascertainment bias notwithstanding, empirical data suggest that divergence between sibling species and conspecific populations is predominantly due to evolution of coding genes, independent of their positions in gene regulatory networks, but morphological differences between species that diverged longer ago are predominantly due to *cis*-regulatory evolution[182]. The following explanation has been offered: Selection acting early during population divergence may partly overcome the negative fitness effects of antagonistic pleiotropy that are expected for coding mutations, but may not be strong enough to fix these mutations[182]. Over time, as more mutations become available, *cis*-regulatory mutations with more specific effects and less antagonistic pleiotropy would replace the coding variants. An interesting implication is that the mutations responsible for phenotypic differences between older species may be distinct from those that are causally important in the process of population divergence and speciation, even when the mechanism of speciation and the diverging phenotypes are the same. Studies of the genomic basis (coding versus regulatory) of species divergence in incipient versus older species in the same taxon are needed to test this hypothesis. We are not aware that such data exist.

These are exciting times for speciation research, and major progress in the field is likely to come from integrating the analyses of genomic data with studies of ecology, behavior, developmental biology and theory. We propose three major building blocks as a roadmap for such continued integration.

First, there is a need for more comparative genome scans at different stages in the speciation continuum in closely related taxa or in replicate species pairs in the same taxon. These data need to be combined with annotation of the effects of alleles on phenotypes and on RI, which can be done through QTL mapping or functional analyses in the context of annotated reference genomes. This would allow the association of divergent genomic regions with mechanisms of RI. Such studies need to be repeated in the following scenarios: in taxa in which speciation is driven by ecology, sexual selection and intrinsic incompatibilities (Box 2); in different spatial contexts; and in taxa that have not speciated, but that occupy similar environments to those taxa that have undergone speciation. Sampling design should explicitly aim to explore variation, both in different stages on the speciation continuum and for different degrees of geographical isolation (Fig. 2), and the history of geographical isolation should ideally be known. Eventually, with replication and clever experimental and comparative study designs, it will become possible to understand whether different mechanisms and modes of speciation can be distinguished based on patterns observed in genome-wide data.

Second, experimental **population genomics** studies of speciation are needed to measure the strength and multifarious nature of selection, and more generally to test hypotheses about processes underlying differentiation and isolation, including intragenomic conflict, heterogeneity in recombination rates, and coupling.

Third, theoretical modeling is needed that includes the influences of variable demography, recombination rates and time, and explicitly considers standing genetic variation and different sources of incompatibilities. Such models will be helpful in generating predictions that can be tailored to individual empirical study systems to make them testable. Such predictions could include genomic signatures of alternative speciation modes and mechanisms, and how modes and mechanisms can be inferred from patterns found in genomes at different stages of the speciation continuum. Improved methods for estimating the timing of long-term gene flow would also be very valuable[90]. Given the increasingly widespread evidence for recruitment of ancient genetic variation into recent speciation events, analytical methods for rigorous hypothesis-testing regarding the source of such variation – that is, the contributions of

hybridization and standing genetic variation – are also needed. Such methods could include comparisons of the phylogenetic histories of genomic regions that confer adaptation and reproductive isolation with those of other segments of the genomes of young sister species[74, 77, 99, 112].

Conclusions

New approaches for gathering large amounts of genomic data in non-model organisms have begun to produce intriguing and unexpected insights into the genetics of speciation. Sympatrically coexisting species are characterized by heterogeneous differentiation that is widely scattered across the genome even when these species are still very young, but adaptive differentiation between parapatric populations can be restricted to a few genomic islands. Ancient alleles with large and pleiotropic effects characterize both types of divergence, and were often acquired by interspecific hybridization. Genomic conflict may be a frequent source of intrinsic postzygotic isolation. It may be recognized in genome scans as strong sweep signatures on sex chromosomes or in isolated islands of divergence on autosomes. More strongly integrated studies are now needed that cover multiple components of RI at multiple stages of the speciation continuum, and in geographical settings ranging from complete allopatry to full sympatry, paying additional attention to the history of population contact (primary or secondary). With the rapid growth of genomic data generation and analysis approaches, it will then soon become possible to construct an integrated picture of speciation starting from the evolution of reproductive barriers and how this is influenced by ecological and genomic constraints, through the way speciation creates signatures of genomic divergence, to how genomic properties of organisms interact with history and ecology in shaping patterns in biodiversity. There is no doubt that a new phase of discovery has begun that will usher in a greatly increased understanding of the origin of species.

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Glossary

Items included in the glossary are bolded in their first appearance in the main text.

Admixture mapping

Identification of genetic loci that contribute to phenotypic differences between ancestral populations, by exploring genotype-phenotype correlations in a population of mixed ancestry.

Allopatric

Organisms, populations or species inhabiting distinct geographical regions and therefore not exchanging genes.

Allopatry

Occurrence in geographically isolated regions.

Cline

Directional variation in phenotype or genotype, or change in frequency (e.g. of an allele), across a geographic region.

Coalescence

The merging of two genetic lineages in a common ancestor.

Coalescent

A statistical framework for the analysis of genetic data where the genotypes shared by populations or species are traced back in time to their most recent common ancestor.

Correlational selection

Selection for optimal character combinations.

Disruptive selection

Selection within a single population that favours extreme phenotypes over intermediate phenotypes.

Distorter loci

Loci underlying **meiotic drive**, the non-Mendelian segregation of alleles in meiosis. Distorter loci may act on other loci, so-called responder loci.

Divergence hitchhiking (DH)

Occurs when divergent selection on a locus reduces the effective migration rate for physically linked regions, which increases the opportunity for divergence at loci under weaker selection in these surrounding regions. DH regions may remain much larger than traditional hitchhiking regions after a selective sweep within populations because of the persistent reduction in the ability of flanking regions to recombine away from a divergently selected gene.

Divergent selection

Selection favouring different phenotypes in different populations.

D_{xy}

The average number of nucleotide substitutions per site between two populations.

Bateson-Dobzhansky-Muller Incompatibility (BDMI or mostly just referred to as DMI)

An intrinsic postmating barrier that is the result of epistatic interactions between alleles at two or more loci that cause reduced fitness in hybrids but not in the parental populations.

Ecological speciation
The evolution of reproductive isolation as a consequence of divergent or disruptive natural selection between populations that inhabit different environments or exploit different resources.

Ecotone
A zone where there is a transition between two distinct biological communities, e.g. between forest and grassland or aquatic and terrestrial habitats. Ecotones are typically associated with changes in the physical environment.

Extrinsic reproductive isolation
Fitness reduction in hybrids that is dependent on the environment, i.e. mediated by genotype-environment interactions.

Fixation
Describes the situation in which a mutation or variant has achieved a frequency of 100% in a population.

F_{ST}
A measure of population subdivision that compares the correlation between two gene copies that are randomly drawn from the same population to that between two gene copies drawn from two different populations. An F_{ST} of 1 indicates that two populations are fixed (**fixation**) for alternative alleles.

F_{ST}-outlier analysis
Comparison of the distribution of F_{ST} values across loci with the distribution expected in the absence of divergent selection for the same average differentiation. Loci whose F_{ST} values exceed expectation are likely to be influenced by divergent selection, either on the locus itself or on a linked locus.

Gene flow
The movement of alleles between populations. For gene flow to occur, individuals must disperse between populations and successfully reproduce with local individuals. Therefore, gene flow can be reduced not only by dispersal barriers but also by intrinsic or extrinsic reproductive isolation.

Gene-flow-selection balance
A level of differentiation between sub-populations at which the homogenizing effect of gene flow and the differentiating effect of divergent selection are in equilibrium.

Genome scan
Comparison of genome-wide patterns of diversity within populations and/or divergence between populations at hundreds or thousands of markers. Most studies until recently used Amplified Fragment Length Polymorphisms (AFLPs) but this has recently changed, and SNPs generated by NGS or SNP chips are being used.

Genomic conflict
Genomic conflict arises between genes or genetic elements within the same genome when these are not transmitted by the same rules (e.g. biparental vs uniparental inheritance), or when a gene causes its own transmission to the detriment of the rest of the genome. The presence of elements (distorter loci) that bias transmission is expected to lead to the evolution of loci that restore Mendelian segregation (restorer loci).

Genomic coupling
The statistical association between different traits and loci involved in RI.

G-matrix
The additive genetic variance-covariance matrix that summarizes the variances within and covariances between multiple phenotypic traits.

Hybridization
Mating between individuals that belong to distinct species or populations. If postmating isolation is incomplete, hybridization leads to the introgression of genes from one population to the other.

Hybrid zones
Spatially restricted regions where the distribution ranges of distinct populations or incipient species come into contact and hybrids are formed.

Incomplete lineage sorting
Situation in which some alleles share a more recent common ancestor with alleles in another species than with other alleles in the same species.

Intragenomic conflict
Antagonistic selection among genomic elements with different fitness interests in an individual.

Intrinsic reproductive isolation
Fitness reduction in hybrids that is independent of the environment.

Introgressive hybridization
The introduction of genes from one population or species into another through hybridization.

Linkage disequilibrium
The statistical association of the alleles at two loci within gametes in a population. Although linkage disequilibrium tends to be greater between linked loci, it can also arise between physically unlinked loci — for example, because of selection, non-random mating or gene flow.

Locus or allele effect size
The magnitude of the influence of a locus, or a specific allele, on a phenotypic trait. This can be expressed, for example, as the proportion of phenotypic variation attributable to a specific locus or the phenotypic difference between genotypes with and without a specific allele.

Multifarious divergent selection
Divergent selection acting on multiple traits.

Multiple-effect traits or “magic” traits
Traits that contribute to more than one component of reproductive isolation, such as a trait contributing to local adaptation that is also used as a mating cue.

Meiotic drivers
Factors distorting Mendelian segregation. At a heterozygous site, the driving variant will be found in more than half of the gametes.

Next Generation Sequencing
A class of high-throughput sequencing methods that rely on technologies that parallelize the sequencing process, producing thousands or millions of sequences concurrently. Next Generation Sequencing technologies increase throughput and lower the cost of DNA sequencing by orders of magnitude compared to standard dye-terminator methods.

One-allele mechanism
Reproductive barriers arise through spreading of the same allele in each of two diverging populations, such as an allele for behavioural imprinting or reduced migration.

Parapatric
Organisms, populations or species that inhabit adjacent geographical regions or spatially distinct but adjacent habitats and may exchange genes.

Pleiotropy
Effect of an allele on more than one trait.

Prezygotic isolation
Effect of barriers acting before or after mating but before fertilisation, including the isolating effects of divergent mate choice, habitat preference, reproductive timing and gametic incompatibility.

Population genomics
Use of genome-wide data (typically based on next-generation sequencing methods) to make inferences about evolutionary processes in natural populations.

Postzygotic isolation
Effects of barriers acting after fertilisation, such as hybrid sterility and hybrid inviability. Can be extrinsic (mediated by the environment) or intrinsic.

Quantitative trait locus (QTL)
Chromosomal region with a statistically significant effect on a phenotype.

Reinforcement
Selection for the strengthening of prezygotic barriers to avoid the production of unfit hybrids between taxa that have previously evolved some postzygotic isolation.

Reproductive isolation
Absence or restriction of gene flow between populations over and above that due to spatial separation alone.

Responder loci
Loci showing deviations from Mendelian segregation (meiotic drive) due to the effect of a distorter locus.

Secondary contact
The meeting of the distibution ranges of two distinct populations or species after a period of evolutionary divergence in geographical isolation (allopatry).

Sexual conflict
The evolution of phenotypic characteristics by sexual selection, when the trait confers a fitness benefit to one sex but a fitness cost to the other.

Sexual isolation
Reproductive isolation as a consequence of reduced mating between members of divergent populations, including behavioural assortative mate choice and assortative fertilization in animals, as well as pollinator-mediated assortative mating in plants. Most often thought of as prezygotic, but can be postzygotic if there is disruptive sexual selection.

Speciation continuum
Pattern where the strength of reproductive isolation between two incipient species varies in different locations or in different species pairs that belong to the same evolutionary lineage and diverge in similar ways.

Speciation genomics
The field of speciation research that addresses the influence of genomic properties on the evolution of reproductive barriers and the signatures of speciation processes that are observable in genomic patterns, for example of diversity and divergence. Its aim is a conceptual and methodological integration of genomic approaches with other empirical and theoretical speciation research.

Standing genetic variation
Allelic variation that is currently segregating within a population; as opposed to alleles that arise through new mutation events.

Sweep
Increase in frequency of an allele and closely linked chromosomal segments due to positive selection. Sweeps initially reduce variation and subsequently lead to a local excess of rare alleles as new unique mutations accumulate.

Sympatric
Organisms, populations or species that share the same geographical region and overlap in their use of space with no spatial barriers to gene exchange.

Transgressive phenotypes
Expression of phenotypic variation in hybrids that exceeds the range of phenotypes observed in the parental taxa.

Two-allele mechanism
Reproductive barriers arise through spreading of different alleles at the same locus in two diverging populations, such as alleles for different habitat or mating preferences.

Underdominance
Heterozygote inferiority. The phenotype expressed in heterozygotes has lower fitness than that of either homozygote. Underdominance can be a cause of **disruptive selection**.

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Box 1: Genomic tools for studying speciation

Next-generation sequencing (NGS) is rapidly expanding the tool box available for studying speciation.

Patterns of genomic divergence: Several methods can be used to investigate genome-wide divergence along the speciation continuum. These methods include: genome scans using SNP arrays[78], RAD-seq[72, 77] or related genotyping-by-sequencing (GBS) methods, whole exome or transcriptome sequencing[76] and whole genome re-sequencing[113] of population samples.. Patterns in genome-wide divergence can be visualized and compared by means of F_{ST} kernel density plots (Fig. 2) and Manhattan plots[98].

Testing for signatures of introgression: Various approaches are available to assess if genetic variants are shared between incipient species as a result of hybridization or due to incomplete lineage sorting[90]. The ABBA-BABA test[184] is particularly applicable to genome-scale datasets. It relies on the frequencies of two specific patterns of allele sharing among a group of four species.

Identifying signatures of selection: Genome scans can reveal genomic regions that show evidence of divergent selection between incipient species using **F_{ST}-outlier analysis** or related approaches, which can be applied to individual SNPs[77] or to smoothed average F_{ST}s[72] within windows or regions of the genome. The latest methods can account for demographic and other sources of variation (e.g. [105, 185]) and make improved use of high-density marker information[186].

Mapping genes that are involved in reproductive isolation: Genome scans of incipient species pairs along the speciation continuum are a logical first step in the search for candidate RI genes[69, 72, 74, 98]. A range of genetic mapping tools are available to identify links between divergent genomic regions and the phenotypic traits that contribute to RI. **Quantitative trait locus (QTL)** mapping is one powerful such method[187]. In short, a genome-wide set of markers is genotyped in a phenotypically variable population with known pedigree to statistically associate markers (QTLs) with phenotypes of interest (in this case traits associated with RI). With functional information on genes in the vicinity of a QTL, candidate RI genes can be identified.

Admixture mapping: If pedigree data are not available, it is possible to take advantage of the phenotypic and genetic differences that exist between hybridizing taxa and use admixture as the basis for genetic mapping of phenotypes that contribute to RI[109, 188] using samples from wild hybrid populations. Intrinsic and extrinsic postzygotic barriers involve alleles that are selected against in hybrids and a variety of methods can be used to identify such alleles in hybrid zones or in other situations where admixture occurs. Genomic cline analysis[189] is one such method in which candidate RI loci with low levels of introgression relative to most of the genome can be identified[79, 190].

Manipulative selection experiments: QTL and admixture mapping have an unfortunate bias toward detecting loci of large effect[148]. Alternatively, alleles affecting fitness and RI can be located using manipulative selection experiments which track allelic changes or genome-wide responses[86, 191]. Estimates of these effects can be ascertained by measuring selection and introgression in the wild. To date very few studies have taken this approach and none has measured effects on reproductive isolation.

Gene expression studies: To further investigate the significance of candidate RI-loci, expression QTL (eQTL) analysis can be useful. It identifies genomic loci that regulate expression levels of mRNAs[192]. Systematically generated eQTL information can provide insight into the mechanism underlying reproductive isolation in regions identified through genome-wide association studies, and can help to identify networks of genes and the role of gene interaction (including epistasis in DMIs) in reproductive isolation.

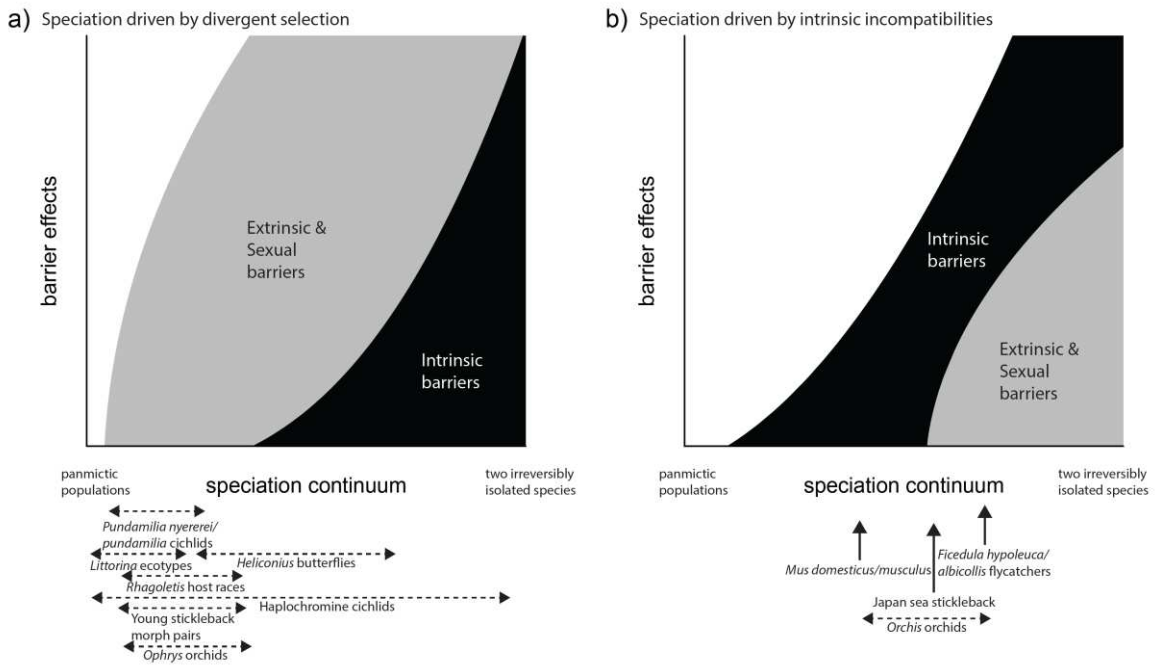
Box 2. Evolution of reproductive isolation

Reproductive isolation (RI) can usefully be divided into three forms: i) Extrinsic forms of postzygotic isolation result from divergent ecological or sexual selection and depend on interaction with the environment or with other individuals (e.g. reduced viability or fertility of migrants and hybrids due to ecological or behavioral factors). ii) Intrinsic forms of postzygotic isolation are due to environment independent genetic incompatibilities (e.g. Bateson-Dobzhansky-Muller incompatibilities). iii) Finally, prezygotic isolation includes phenological isolation, habitat isolation, and sexual isolation due to assortative mating or fertilization.

In speciation driven by divergent ecological or sexual selection, extrinsic and prezygotic forms of isolation evolve first, and often interact, to produce reproductive isolation, and intrinsic forms of isolation will often only evolve later in the speciation process (Panel A). In contrast, speciation driven by intrinsic barriers often results from epistatic incompatibilities, which may (though do not necessarily[19]) accumulate in an accelerating "snowball" fashion[61, 193] as a by-product of selection or due to genetic drift (the latter only slowly). Extrinsic postzygotic and prezygotic barriers may accumulate later, facilitating ecological coexistence between sibling species and reinforcement of reproductive isolation (Panel B).

In both panels the x-axis depicts the position of a diverging taxon pair on the "speciation continuum" (in terms of relative time) and the y-axis represents the strength of reproductive isolation (RI) between sister taxa. Curve shapes are hypothetical, and reflect the idea that in speciation driven by divergent selection, extrinsic postzygotic and sexual barriers arise rapidly early in speciation. Classes of barriers within each panel are not necessarily additive or interactive, and the emergence of RI via either of these barrier types should be viewed as independent trajectories. Movement along the speciation continuum, from weakly isolated species to irreversibly isolated ones, is not constant, speciation can go back and forth, or be arrested at intermittent stages, and the average timescales for speciation via the processes contrasted here (Panels A & B) may vary.

Arrows along the x-axis indicate the position(s) of model systems (studied by the authors of this paper) along the speciation continuum. These organisms vary in the strength and types of barriers isolating incipient and sister species. Studies of the genomics of speciation at different points on the speciation continuum are emerging in several systems, mainly where speciation is driven by divergent selection (as indicated by the dashed arrows showing timespans along the speciation continuum). In many cases strong reproductive isolation may never evolve, particularly in ecological speciation (e.g. [122]). Incomplete reproductive isolation may facilitate cases of "speciation reversal" (e.g.[194]) and "ephemeral" speciation (e.g. [195]).



Box 3: New data for new theory: speciation genomics and patterns in biodiversity

As speciation produces the raw material for biodiversity patterns, connecting speciation processes to these patterns in biodiversity is an important goal[196]. We envisage that speciation genomics can make important and unique contributions to elucidating these connections. Study of the distribution of species richness among clades provides evidence for non-uniform diversification rates among taxa, which can arise from differences in speciation and/or extinction rate (e.g.[197]). Speciation rates estimated from the fossil record are far slower than those predicted from mathematical models and observed in studies of recent diversification, and one explanation for this discrepancy is a high frequency of "ephemeral speciation," in which taxa that have recently undergone speciation have high rates of extinction[195]. This has been documented in cases of "speciation reversal"[194, 198, 199] which is possible when speciation does not reach "completion"[122, 200].

A better understanding of the genomic basis of speciation might help us to understand the influence of speciation on species persistence and patterns of species diversity. For instance, ecological speciation readily and rapidly produces divergent, partially isolated ecotypes and species that may immediately be able to coexist without competitive exclusion. Ecological speciation might thereby contribute disproportionately to the buildup of biodiversity compared to non-ecological mechanisms[196]. However, isolation between young ecologically differentiated species is often extrinsically based and contingent upon the persistence of divergent selection (see Box 2). The species that arise most rapidly may therefore be those species that are most vulnerable to extinction early in their histories[200] . In contrast, speciation via intrinsic mechanisms may produce species that are less prone to ephemerality because speciation reversal may be less likely. However, speciation rates might be slower in these lineages than in lineages where ecological speciation is common, and ecological differences must evolve after speciation in order for closely related taxa to coexist. Progress in connecting speciation to broader-scale patterns of species richness will require attention to how speciation mechanisms, and their genomic basis, influence rates of speciation and the persistence and coexistence of young species. If mechanisms of speciation leave distinctive genomic signatures, correlation between genomic patterns and disparity in species richness among clades could be tested quantitatively using comparative phylogenetic approaches.

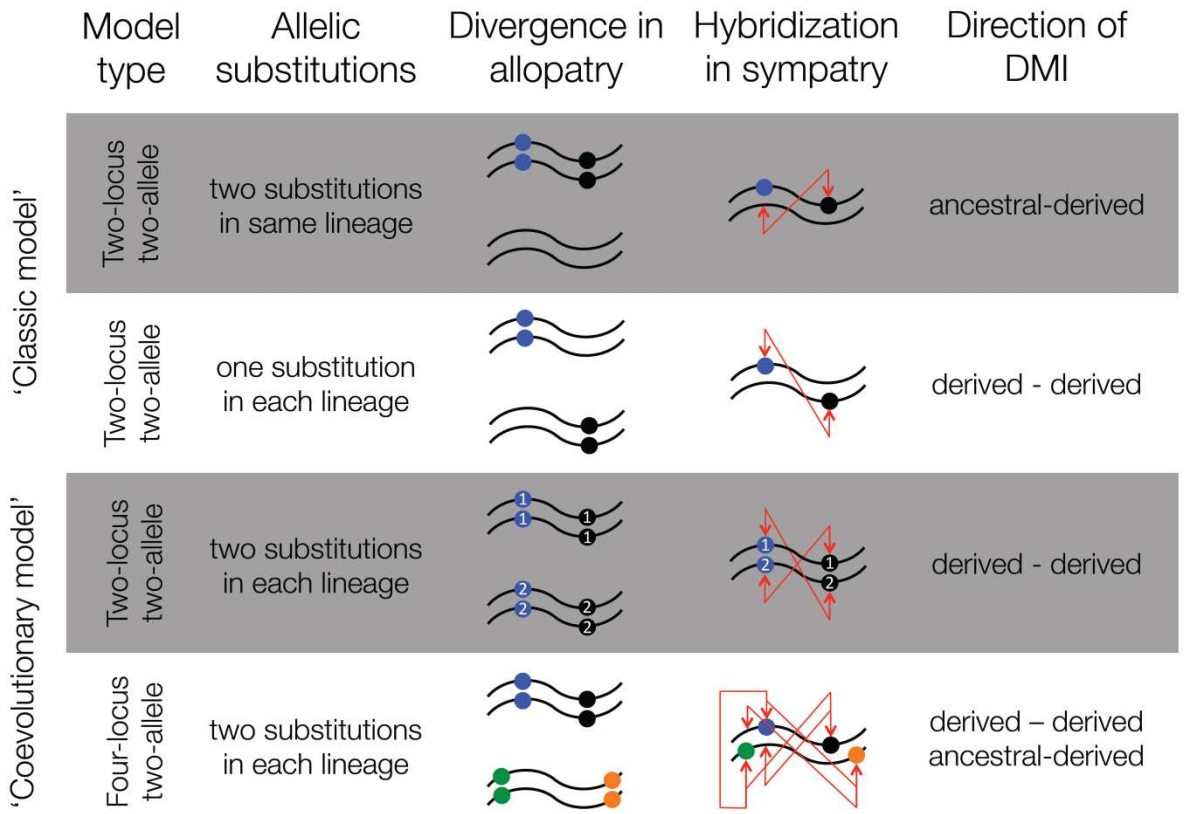


Fig. 1. 'Classic' and coevolutionary models of hybrid incompatibility in a genomic conflict scenario. In the 'classic model', Bateson-Dobzhansky-Muller incompatibilities (DMIs) are envisioned as two-locus, two-allele interactions, in which incompatibilities arise between an ancestral allele and an allele derived in one lineage (1st row) or between alleles derived in two separate lineages (2nd row); a special case of the latter model can refer to maternal-effect selfish loci in which maternal "poison" and zygotic "antidote" are due to developmental expression divergence of the same locus. In the coevolutionary models, DMIs are continually fixed at the same loci (3rd row) or at different loci (4th row). In all examples with two substitutions in a lineage, the selfish locus (left) drives the evolution of the restorer locus (right). Red arrows indicate negative epistatic interactions between complimentary loci. In all models, the ancestral state is wild-type except for row three. In this row, the ancestral state is a coevolving selfish element-restorer system. Insight into the role of genomic conflict in speciation reveals the potential for further development of models of hybrid incompatibility. Models that incorporate the possibility for increased lag-load due to ongoing coevolution predict successively more severe incompatibilities. Additional theoretical work is needed to investigate such coevolutionary models.

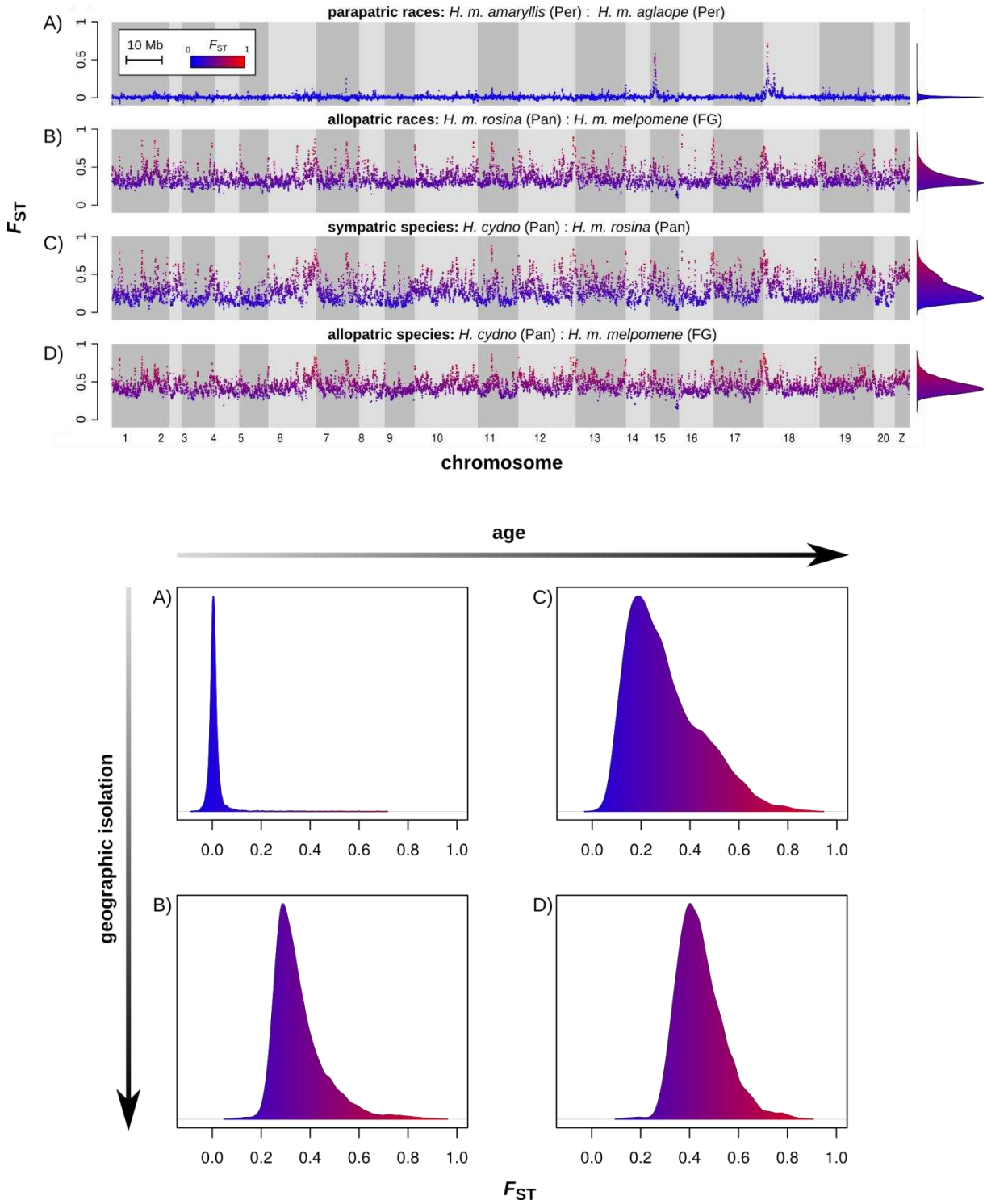
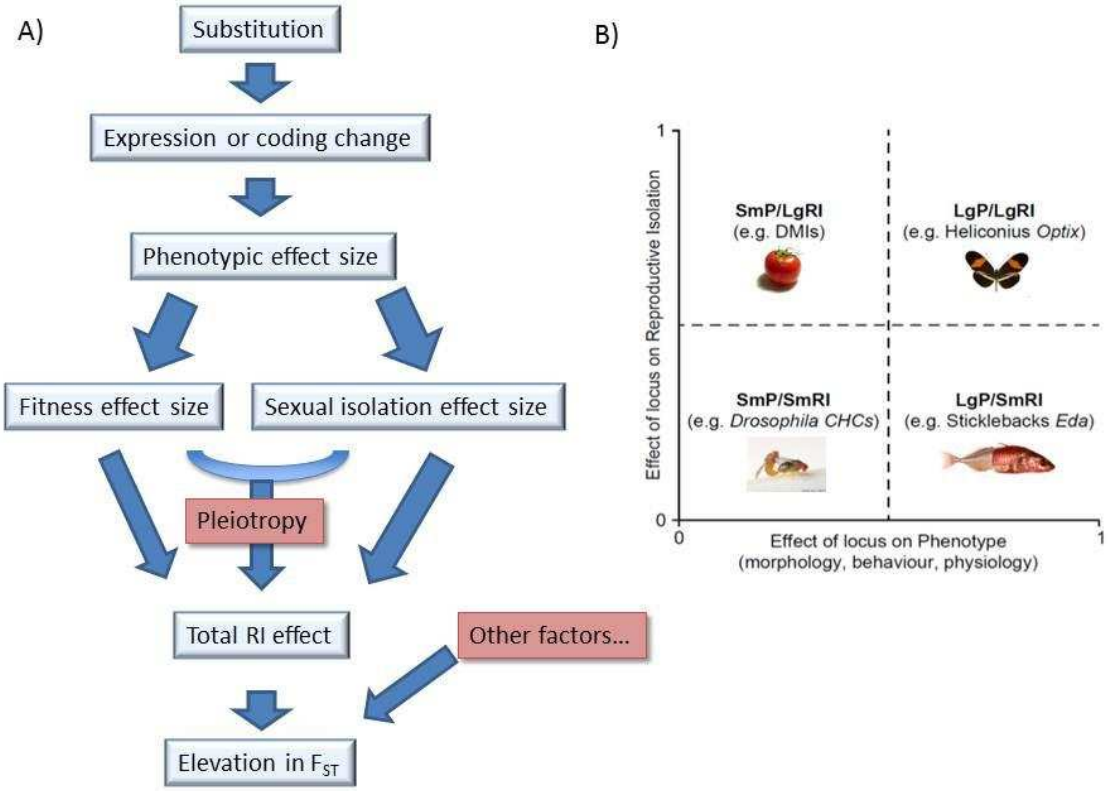


Fig. 2. **Genomic patterns of divergence along the speciation continuum in *Heliconius* butterflies**. The top panel shows the patterns of differentiation between hybridizing parapatric races (A) and sympatric species (C) and between geographically isolated races (B) and species (D) along the genome. Divergence is highly heterogeneous even between allopatric populations of the same species (B). The shape of the frequency distribution of locus-specific F_{ST} values (bottom panel) clearly differs between the different stages in the continuum and between geographic scenarios with, for example, the greater variance in (C) consistent with gene flow between species in sympatry. However, the challenge is to distinguish between speciation with (A, C) versus without (B, D) gene flow.



1400

1401 **Fig. 3. Effect sizes of substitutions on phenotype and on reproductive isolation.** (A) Effects of variation
1402 at different levels, and connections between those levels. The size of effect can vary at each step from zero or
1403 quite small to very large. A substitution can alter gene expression or protein coding, which in turn has some
1404 effect on a phenotype. This phenotype can have effects of varying size on environment-dependent fitness (and
1405 hence possibly extrinsic postzygotic isolation), environment-independent fitness (hence possibly intrinsic
1406 postzygotic isolation) and on prezygotic isolation. Alternatively a phenotype may pleiotropically affect both
1407 fitness and prezygotic isolation. All these effects combine to generate total RI, which will likely elevate F_{ST},
1408 although other factors can alter F_{ST} as well. (B) The lack of correlation between the effect of a locus on
1409 phenotype (P) and on reproductive isolation (RI). An example for each of the four relationships is shown to
1410 illustrate that phenotypic effect size does not necessarily predict RI effect size: loci with small effect on
1411 phenotype and large effect on reproductive isolation (SmP/LgRI: DMIs in *Solanum*[27]); loci with large effect on
1412 phenotype and large effect on reproductive isolation (LgP/LgRI: *Optix* in *Heliconius* [160]); loci with small effect on
1413 phenotype and small effect on reproductive isolation (SmP/SmRI: CHCs in *Drosophila*[201]); loci with large effect
1414 on phenotype and small effect on reproductive isolation (LgP/SmRI: *Eda* in stickleback[191]). The relationships
1415 between phenotypic and RI effect size and F_{ST} are largely unknown at present.

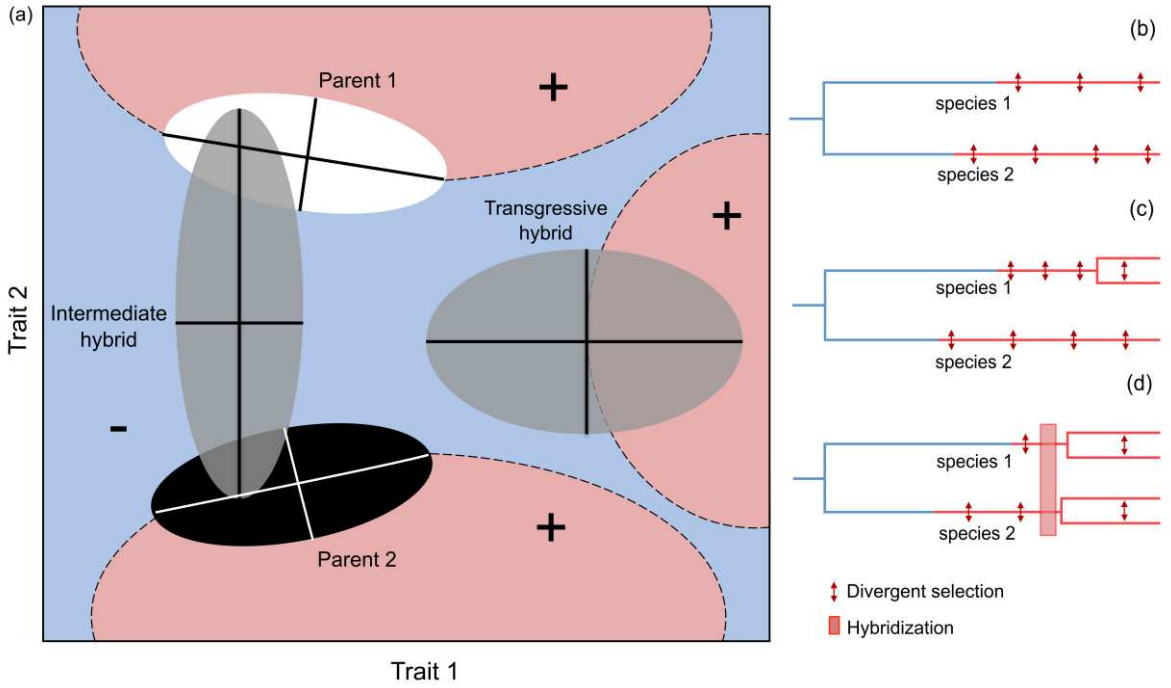


Fig. 4. Influence of genetic constraints on speciation. (A) With the help of NGS, it is now feasible to infer relatedness of individuals in any given natural population and thus to estimate a **G**-matrix without the use of pedigree-data[173]. The **G**-matrix (represented here as an ellipse in the space of two quantitative traits) can bias evolution in certain directions, and depending on the adaptive landscape (represented by regions of higher (+; red) and lower (-, blue) fitness than the parental populations (white, black)), might constrain adaptive divergence and speciation. Hybridization events may facilitate speciation by aligning the **G**-matrix in the direction of divergence between parental species (intermediate hybrid), or by giving rise to novel phenotypes (transgressive hybrid) in new regions of positive fitness that cannot be reached through gradual evolution in either of the parental species.

(B-D) The influence of genetic constraints on speciation can be tested at the phylogenetic level. (B) Constraints may persist over evolutionary time as a result of the inability of divergent selection to change genetic architecture, preventing speciation from happening. (C) Alternatively, other forms of selection (e.g. correlational selection) can alter the structure and orientation of the **G**-matrix and potentially facilitate divergence and speciation over moderate time scales. (D) Hybridization and gene flow can dramatically alter **G** in just a few generations, fueling adaptive divergence and resulting in sudden bursts of speciation. Note that hybridization between sister species is shown here for illustration, but hybridization that facilitates divergence may occur more widely among related taxa.

Biographies

Ole Seehausen studied speciation and hybridization since his PhD at the University of Leiden in the 1990s. Adaptive radiations receive his particular attention, such as the cichlid fishes of Lake Victoria and, more recently, the whitefish of prealpine European lakes, stickleback and trout. He is a professor in the Institute of Ecology & Evolution of the University of Bern and head of a research department at EAWAG, the Swiss Federal Institute of Aquatic Science and Technology. His lab combines ecological and behavioral research with genetics and genomics to investigate processes and mechanisms implicated in adaptation, speciation, species coexistence and extinction.

Roger Butlin has studied speciation since his postdoctoral work with Godfrey Hewitt in the 1980s. He is interested in the processes generating reproductive isolation and its genetic basis. Reinforcement has been a particular focus of study. Current projects are examining the role of chemosensory genes in aphid host race formation and the genetic basis of parallel local adaptation and speciation in periwinkles. He is a professor of evolutionary biology at the University of Sheffield in the UK and currently holds the 2013 Tage Erlander guest professorship at the University of Gothenburg in Sweden.

Irene Keller is a bioinformatician at the Department of Clinical Research of the University of Bern (Switzerland). She received her PhD from the University of Bern and worked as a postdoctoral fellow with Richard Nichols at Queen Mary University of London and with Jukka Jokela and Ole Seehausen at Eawag and University of Bern (Switzerland). Her interests focus on the application of molecular and bioinformatics tools to understand the genetic basis of adaptation, speciation and human disease.

Catherine E.Wagner is an evolutionary biologist with interests in speciation and the origins of diversity, and the relationships between diversity-generating processes and macroevolutionary patterns. Her research uses population genetic, phylogenetic, and comparative methods to study diversification. She is currently a postdoctoral researcher at Eawag, the Swiss Federal Institute of Aquatic Science and Technology and the University of Bern, Switzerland, where her work focuses primarily on African cichlid fishes. She earned her Ph.D. in ecology and evolutionary biology from Cornell University in 2011.

Janette Boughman and her lab study the selective forces causing speciation in threespine sticklebacks, with particular focus on sexual selection and its interaction with natural selection to generate reproductive isolation. She uses lab and field behavioral experiments to understand the subtle yet powerful action of these forces on phenotypic and genetic evolution and how this transmits to the genome. She has studied both the accumulation of reproductive isolation and its loss through reverse speciation. Recent work investigates fitness landscapes at both the phenotypic and genetic level and their role in diversification. She is Associate Professor at Michigan State University.

Paul A. Hohenlohe is an Assistant Professor in the Department of Biological Sciences and the Institute for Bioinformatics and Evolutionary Studies at the University of Idaho. He earned his Ph.D. in zoology at the University of Washington in 2000 and subsequently worked as a conservation biologist and postdoctoral researcher. His research focus is on evolutionary genetics and genomics, including RAD sequencing and other tools for population genomics and conservation in non-model organisms, experimental evolution, and evolutionary quantitative genetics theory.

Catherine Peichel earned her Ph.D. in the area of developmental genetics at Princeton University in 1998. During this time, she became intrigued by the genetic basis of phenotypic differences between species. Thus, during her postdoctoral fellowship with David Kingsley at Stanford University, she helped to develop the threespine stickleback as a genetic and genomic model system. She has led a research laboratory at the Fred Hutchinson Cancer Research Center in Seattle, Washington since 2003. Her lab takes a number of approaches to investigate the genetic and genomic changes that underlie adaptation and speciation in sticklebacks.

Glenn-Peter Sætre is a professor in evolutionary biology at the University of Oslo, Norway. He obtained his doctorate also in Oslo and worked several years at Uppsala University, Sweden as a post doc and assistant professor before returning to Oslo in 2003 as full professor. He studies speciation, hybridization and adaptive evolution, mainly in birds, combining genomic analysis and population genetics with behavioural and ecological studies. His research lab is currently mainly focusing on the genomics of hybrid speciation.

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Online key points:

- Speciation is a central process in evolution that is fundamentally about the origin of reproductive isolation. The latest generation of genomic approaches provides remarkable opportunities to describe speciation and learn about speciation mechanisms.
- Genome scans, now truly genome-wide and at base-pair resolution, reveal substantial genomic divergence among incipient species even in the face of gene flow, with extensive genomic heterogeneity in the extent of differentiation, especially at early stages of speciation, both in sympatry and in allopatry.
- The sources of this heterogeneity remain incompletely understood. Combining genome scans with sophisticated population genetic modeling, QTL, and admixture analysis has the potential to isolate the influence of selection from demographic, historical and structural effects and to link these sources of genomic divergence to phenotypes and to reproductive isolation.
- Available empirical data suggest that differentiation between parapatric populations can be restricted to few genomic islands, whereas incipient species that coexist in sympatry show differentiation widely distributed across the genome. This may suggest that genomically widespread selection is required to permit the maintenance and perhaps the buildup of genetic differentiation in sympatry.
- Recent genomic studies reveal that the genetic basis of reproductive isolation is often complex. The effects of pleiotropy, genetic correlations, and patterns of recombination need to be considered, alongside effects of ecological and sexual selection as well as genomic conflict.
- A surprising recent discovery has been the re-use of ancient gene variants in speciation, acquired from standing genetic variation or by introgressive hybridization.
- We propose a roadmap for the development of speciation genomics towards answering classical as well as emerging questions in speciation research.